Hiroshima Journal of Medical Sciences
Vol. 33, No. 4, 619~624, December, 1984
HIJM 33-86

The Change of Serum Lipoproteins Observed in Non Insulin Dependent Diabetic Patients and a Study of its Mechanism*)

Kozo HAYASHI, Haruki MATSUMOTO, Kazuro ITO and Noboru OZONO

Department of Internal Medicine, Mitsubishi Mihara Hospital, 3765 Itozaki-cho, Mihara, 729-03, Japan

(Received August 31, 1984)

Key words: VLDL, LDL, Relative body weight

ABSTRACT

The classification of hyperlipoproteinemia observed in twenty-three noninsulin dependent type II diabetic patients who were not controlled well by diet and sulfonylurea was carried out. Four groups were classified (group 1, normal VLDL and normal LDL; group 2, high VLDL and normal LDL; group 3, high VLDL and high LDL; group 4, normal VLDL and high LDL). The most usual hyperlipoproteinemia group was group 3 and the frequency was about 56%. The relationship between abnormality of serum lipoprotein and some factors such as insulin secretion, hemoglobin A_{1c} concentration, lipoprotein lipase activity was studied among three groups (group 1, group 2, group 3). However, it seemed that there was no significant relationship between hyperlipoproteinemia and these factors. The significant difference of relative body weight was observed between group 1 and group 3. This result suggests that there is a significant relationship between hyperlipoproteinemia (high VLDL and high LDL) and obesity observed in type II diabetes mellitus. The reason why obesity caused hyperlipoproteinemia was not cleared, but it seemed that a difference of insulin secretion did not refer to hyperlipoproteinemia observed in type II diabetes mellitus with obesity, because there was no significant difference of insulin secretion between group 1 and group 3.

INTRODUCTION

It is well known that the disturbance of serum lipoprotein is observed in diabetes mellitus. The change is usually manifested as an elevated serum concentrations of very low density lipoprotein (VLDL) and chylomicron in insulin dependent type I diabetes mellitus[1,4]. Lipoprotein lipase activity is regulated by serum insulin[10,13,15] and absolute deficiency of serum insulin in insulin dependent type I diabetes mellitus causes a decrease of lipoprotein lipase activity. Serum VLDL and chylomicron are catalyzed by lipoprotein lipase. Therefore, it is considered that the decrease of lipoprotein lipase activity is related to the elevations of serum VLDL and chylomicron concentrations in insulin dependent type I diabetes mellitus at least in part.

In non insulin dependent type II diabetes mellitus, high concentration of serum VLDL is observed frequently[9], however it is generally considered that lipoprotein lipase activity is not impaired[12]. The mechanism for hyperlipidemia

*) 林 幸三, 松本春樹, 伊藤和朗, 小園 昇：インスリン非依存型糖尿病にみられる血清リポ蛋白の変化とその機序に関する研究

Postal address: Kozo Hayashi Tsuchiya Hospital 3-30 Nakajima-cho, Naka-ku, Hiroshima 733, Japan

Abbreviation: VLDL, very low density lipoprotein; LDL, low density lipoprotein; IRI, immunoreactive insulin; BG, blood glucose; HTGL, hepatic triglyceride lipase; n. s., not significant.

in human diabetes mellitus are complicated and depending on any factors such as the degree of insulin deficiency, obesity, heredity and diet. Many reports have described the comparison between healthy controls and diabetic patients. In the present study, diabetic patients not well controlled (fasting blood glucose concentration was more than 180 mg/dl) were examined, and abnormality of serum lipoprotein observed in non insulin dependent type II diabetes mellitus was classified and the relationship between serum lipoprotein disturbance and some factors such as insulin secretion, hemoglobin A_{1c} concentration, lipoprotein lipase activity and relative body weight was investigated.

MATERIALS AND METHODS

Subject

Twenty-three non insulin dependent type II diabetes mellitus patients (eleven males and twelve females), ranging in age from 45 to 65 years old were studied. Diabetes mellitus was diagnosed on the basis of fasting blood glucose, 75g OGTT and other clinical signs. Diabetic patients studied were treated by diet and sulfonylurea but not controlled well and their fasting blood glucose concentrations were more than 180 mg/dl. All tests were started after a fasting of 12 hr and before administration of drugs.

Measurement of lipoprotein

Measurements of VLDL and LDL were carried out by heparin-Ca^{2+} sedimentation method in Special Reference Laboratories, Inc. Briefly described, three samples of serum were mixed with 2 ml of solution containing 10 mg heparin, 2 ml of solution containing 10 mg heparin and 1.2 g sodium chlorde or 2 ml of solution containing 10 mg heparin and 1.8 g sodium chloride at 25°C. Absorbance of 650 nm wave length for each samples was measured after 25 min and concentrations of VLDL and LDL were calculated with the equation determined by Special Reference Laboratories, Inc.

Assay of lipoprotein lipase activity

Postheparin plasma was obtained 10 min after a rapid intravenous injection of 10 IU of heparin per kg of body weight. Blood was collected into tubes containing 5 IU of heparin per ml of blood and kept on ice. Plasma was separated by centrifugation at 4°C and stored frozen at −20°C. 250 μl of plasma was mixed with 50 μl of 0.2 M Tris-HCl buffer (pH 8.4), 50 μl of 20% human serum albmin (pH 8.4), 100 μl of 5 M NaCl solution and 50 μl of activated intralipid solution for hepatic triglyceride lipase activity and was incubated for 1 hr at 37°C. After indicated incubation period, 50 μl of reaction solution mixed above was collected and released non esterized free fatty acid was measured by a modification of Dumcombe's method[5]. When total lipoprotein lipase activity was measured, 100 μl of 5 M NaCl solution in reaction solution was replaced by 100 μl of double distilled water. Extra hepatic triglyceride lipase activity was calculated from the difference between total lipoprotein lipase activity and hepatic triglyceride lipase activity. Activation of intralipid solution was performed by addition of the same volume of normal human serum to 10% Intralipid solution and incubation for 30 min at 37°C.

Other methods

Plasma glucose was measured by glucose autoanalyzer, plasma insulin by radioimmunoassay and hemoglobin A_{1c} by high pressure liquid chromatography. The relative body weight was calculated by a modification of Broca's method as shown following. Relative body weight = 100 × body weight(kg)/(height(cm)−100) × 0.9

Chemicals

All reagents were of the highest purity commercially available, Human serum albmin was obtained from Nihon Syoji Co.. Intralipid was obtained from Midori Juji Co.. Insulin radioimmunoassay kit was obtained from Daiichi Radioisotope Co..

RESULTS

Classification of hyperlipoproteinemia observed in non insulin dependent type II diabetes mellitus patients

Normal concentration of fasting VLDL was determined from 0 to 110 mg/dl and that of fasting LDL was determined from 145 to 455 mg/dl. When the classification of hyperlipoproteinemia was carried out according to the standard values determined above, four groups were classified. The first was normal VLDL and normal LDL group, the second was high VLDL and normal LDL group, the third was high VLDL and high LDL group and the fourth was normal VLDL and high LDL group. The most usual hyperlipoproteinemia was high

Table 1. Classification of hyperlipoproteinemia observed in twenty-three non insulin dependent type II diabetic patients and serum VLDL and LDL concentrations

	VLDL concentration (mg/dl)	LDL concentration (mg/dl)
Group 1 (n=6)		
normal VLDL	87.0±30.9	393.8±56.2
normal LDL		
Group 2 (n=3)		
high VLDL	178.7±17.0	334.3±104.7
normal LDL	(P_1<0.01)	(P_3 n. s.)
Group 3 (n=13)		
high VLDL	229.2±95.8	574.9±104.9
high LDL	(P_2<0.01)	(P_4<0.01)
Group 4 (n=1)		
normal VLDL	61.0	566.0
high LDL		

Serum VLDL and LDL concentrations were determined as described in Materials and Methods. Each value represents mean±S. D. P_1 refers to the t-test for differences of VLDL concentration between group 1 and group 2. P_2 refers to that of VLDL concentration between group 1 and group 3. P_3 refers to the t-test for differences of LDL concentration between group 1 and group 2. P_4 refers to that of LDL concentration between group 1 and group 3.

VLDL and high LDL group, and the frequency was about 56% (Table 1). VLDL and LDL concentrations of group 1 were 87.0±30.9 mg/dl and 393.8±56.2 mg/dl respectively. Those of group 2 were 178.7±17.0 mg/gl and 334.3±104.7 mg/dl respectively. Those of group 3 were 229.2±95.8 mg/dl and 574.9±104.9 mg/dl respectively. As for VLDL concentration, there were significant differences between group 1 and group 2, and between group 1 and group 3. As for LDL concentration, there was a significant difference between group 1 and group3.

Hyperlipoproteinemia and insulin secretion

Insulin secretion (∑IRI) was measured by addition of basal insulin secretion value and insulin secretion values of 30, 60, 120 and 180 min after 75 g glucose loading per os. The difference of insulin secretion was investigated among normal VLDL and normal LDL group (group 1), high VLDL and normal LDL group (group 2) and high VLDL and high LDL group (group 3). Insulin secretions of group 1, group 2 and group 3 were 193.9±138.6, 185.0±67.5 and 170.9±88.4 μU/ml respectively, and there was no significant difference among these three groups. The basal insulin secretions of group 1, group 2 and group 3 were 16.2±8.5, 15.0±2.0 and 23.0±11.7 μU/ml respectively. There was no significant difference of basal insulin secretion among these three groups too. Fur-

Table 2. Hyperlipoproteinemia and insulin secretion

	∑IRI (μU/ml)	⊿IRI/⊿BG in 30 min	basal IRI (μU/ml)
Group 1 (n=6)			
normal VLDL	193.9±138.6	0.143±0.158	16.2±8.5
normal LDL			
Group 2 (n=3)			
high VLDL	185.0± 67.5	0.255±0.224	15.0±2.0
normal LDL	(P_1 n. s.)	(P_3 n. s.)	(P_5 n. s.)
Group 3 (n=13)			
high VLDL	170.9±88.4	0.101±0.133	23.0±11.7
high LDL	(P_2 n. s.)	(P_4 n. s.)	(P_6 n. s.)

Plasma glucose and plasma insulin were determined as described in Materials and Methods. Each value represents mean±S. D. P_1 refers to the t-test for differences of ∑IRI between group 1 and group 2. P_2 refers to that of ∑IRI between group 1 and group 3. P_3 refers to the t-test for differences of ⊿IRI/⊿BG between group 1 and group 2. P_4 refers to that of ⊿IRI/⊿BG between group 1 and group 3. P_5 refers to the t-test for differences of basal IRI between group 1 and group 2. P_6 refers to that of basal IRI between group 1 and group 3.

thermore, the values of ⊿IRI/⊿BG in 30 min were compared among these three groups, but there was no significant difference (Table 2). These results suggest that there is no relationship between hyperlipoproteinemia and insulin secretion.

Hyperlipoproteinemia and hemoglobin A_{1c}

Hemoglobin A_{1c} is known as a marker which reflects blood glucose level before three to six weeks, therefore the condition of diabetic control is suspected by hemoglobin A_{1c} concentration. Hemoglobin A_{1c} concentrations of group 1, group 2 and group 3 were 9.63±3.02, 6.97±1.45 and 9.84±1.96% respectively. In present study, there was no significant difference of hemoglobin A_{1c} concentration among three groups. This result suggests that long duration of hyperglycemia does not have influence on hyperlipoproteinemia such as high VLDL and high LDL (Table 3) and diabetic patients in these three groups were under the same diabetic condition.

Hyperlipoproteinemia and plasma lipoprotein lipase activity

Serum VLDL is produced in liver and catalyzed by lipoprotein lipase which is connected on cell membrane of various tissues in blood stream. Lipoprotein lipase activity were measured separately as hepatic triglyceride lipase activity and extra hepatic triglyceride lipase activity as shown in materials and methods. Hepatic triglyceride lipase activities of group 1, group 2 and group 3 were 821.9±315.8, 1247.5±304.7 and 1236.2±598.9 nmol/ml/hr respectively. Extra hepatic triglyceride lipase activities of group 1, group 2 and group 3 were 5530.2±1386.7, 5282.8±1140.4 and 4750.8±1471.7 nmol/ml/hr respectively. There were no significant differences of hepatic triglyceride lipase activity and extra hepatic triglyceride lipase activity among three groups (Table 4). These results suggest that high VLDL concentrations observed in group 2 and group 3 are not cauesd by decrease of lipoprotein lipase activity.

Table 3. Hyperlipoproteinemia and hemoglobin A_{1c}

	Hemoglobin A_{1c} (%)
Group 1 (n=6)	
normal VLDL	9.63±3.02
normal LDL	
Group 2 (n=3)	
high VLDL	6.97±1.45
normal LDL	(P_1 n. s.)
Group 3 (n=13)	
high VLDL	9.84±1.96
high LDL	(P_2 n. s.)

Hemoglobin A_{1c} was determined as described in Materials and Methods. Each value represents mean±S. D. P_1 refers to the t-test for differences of hemoglobin A_{1c} between group 1 and group 2. P_2 refers to that of hemoglobin A_{1c} between group 1 and group 3.

Table 4. Hyperlipoproteinemia and lipoprotein lipase activity

	HTGL activity (nmol/ml/hr)	Extra HTGL activity (nmol/ml/hr)
Group 1 (n=6)		
normal VLDL	821.9±315.8	5530.2±1386.7
normal LDL		
Group 2 (n=3)		
high VLDL	1247.5±304.7	5282.8±1140.4
normal LDL	(P_1 n. s.)	(P_3 n. s.)
Group 3 (n=13)		
high VLDL	1236.2±598.9	4750.8±1471.7
high LDL	(P_2 n. s.)	(P_4 n. s.)

Plasma lipoprotein lipase activity was determined as described in Materials and Methods. Each value represents mean±S. D. P_1 refers to the t-test for differences of HTGL activity between group 1 and group 2. P_2 refers to that of HTGL activity between group 1 and group 3. P_3 refers to the t-test for differences of extra HTGL activity between group 1 and group 2. P_4 refers to that of extra HTGL activity between group 1 and group 3.

Hyperlipoproteinemia and relative body weight

Relative body weight calculated by a modification of Broca's method described in Materials and Methods of group 1, group 2 and group 3 were 107.7±19.8, 112.0±2.0 and 118.3±20.4 % respectively. When relative body weight was compared, the significant difference was observed between group 1 and group 3, but there was no significant difference between group 1 and group 2 (Table 5). This result indicates that there is a relationship between hyper-

Table 5. Hyperlipoproteinemia and relative body weight

	Relative body weight (%)
Group 1 (n=6)	
normal VLDL	107.7±19.8
normal LDL	
Group 2 (n=3)	
high VLDL	112.0± 2.0
normal LDL	(P_1 n. s.)
Group 3 (n=13)	
high VLDL	118.3±20.4
high LDL	(P_2<0.01)

Relative body weight was determined as described in Materials and Methods. Each value represents mean±S. D. P_1 refers to the t-test for differences of relative body weight between group 1 and group 2. P_2 refers to that of relative body weight between group 1 and group 3.

lipoproteinemia observed in group 3 (high VLDL and high LDL) and obesity.

DISCUSSION

In this study, four groups of hyperlipoproteinemia were classified with serum VLDL and LDL concentrations for type II diabetic patients who were not controlled well by diet and sulfonylurea. Because there was no significant difference of hemoglobin A_{1C} concentration among these three groups, it was considered that these diabetic patients were under the same diabetic condition. An explanation that the deficiency of insulin secretion and insulin action on the peripheral organs cause mobilization of free fatty acid from adipose tissue and overproduction of VLDL in liver is usually accepted in type II diabetes mellitus[11, 12, 14]. The fact that there were no significant differences of basal insulin secretion, $\sum$IRI and ΔIRI/ΔBG in 30 min among group 1, group 2 and group 3 suggests that high VLDL concentration observed in group 2 and group 3 is not caused by insulin secretion. High VLDL concentration observed in obesity is explained by hyperinsulinemia caused by insulin resistance of peripheral tissues and overproduction of VLDL in liver[8], but there was no significant difference of insulin secretion between group 1 and group 3, in spite of the presence of a significant difference of relative body weight between group 1 and group 3 in this study. From these results, it seems that there is no relationship between hyperlipoproteinemia and insulin secretion. For the mechanism of high VLDL concentration, production and catalyzation systems of VLDL must be investigated. Discussions described above are considerations observed by production side. Lipoprotein lipase is the most important enzyme for VLDL catalysis. If lipoprotein lipase activity is decreased in group 2 and group 3, high VLDL concentrations observed in group 2 and group 3 are explained by the activity change of this enzyme. However, no significant difference of lipoprotein lipase activity (hepatic triglyceride lipase and extra hepatic triglyceride lipase activities) was observed (Table 4) as reported previously[12]. This result suggests that overproduction of VLDL causes the increase of VLDL concentration in group 2 and group 3. Other factors rather than insulin secretion seem to contribute to hyperlipoproteinemia in diabetic patients with obesity by means of overproduction of VLDL in liver. One possible conception is the difference of insulin action on peripheral tissues. If insulin action on liver of group 2 and group 3 is larger than that of group 1, overproduction of VLDL in liver and hyperlipoproteinemia may occur, because it is known that insulin accelates to produce VLDL in liver[7]. But this conception was not cleared in this study. Other conceptions such as apoprotein also will be a remarkable event[2].

On the other hand, if diabetes mellitus and hyperlipoproteinemia are independent entities as described by Brunzell et al[3]., it may be meaningless problem to investigate the factors which cause hyperlipidemia of type II diabets mellitus. It is considerable whether there is a relationship between the increase of relative body weight and hyperlipoproteinemia observed in group 3 or not, because there was no significant difference of relative body weight between group 1 and group 2 in spite of the presence of a significant difference of VLDL concentration.

The increase of LDL concentration was observed in group 3. The study of cholesterol metabolism in diabetes mellitus is important to investigate the mechanism of atherosclerotic change. For the mechanism of the increase of LDL concentration observed in diabetes mel-

litus, some conceptions have been suggested. Gonen et al. described the relationship between insulin and LDL receptor, and it is known that specific binding of LDL and LDL receptor is impaired by glycosylation of LDL[6] and Witzum et al. suggest that atherosclerosis is accelated by glycosylation of LDL based on hyperglycemia[16]. In this study, the mechanism for the elevation of VLDL was investigated mainly, but exact mechanism was not cleared. To study the reason why some types of hyperlipoproteinemia are classified in the same condition of type II diabetes mellitus and to clear the mechanism of hyperlipoproteinemia (high VLDL and high LDL) must be continued and the investigation for the problem of involvements of high LDL and high VLDL to atherosclerosis must be carried out.

REFERENCES

1. **Bagdade, J.D. et al.** 1967. Diabetic lipemia. A form of acquired fat induced lipemia. N. Engl. J. Med. **276** : 427-433.
2. **Bar-On, H. et al.** 1976. Serum lipoproteins and apolipoproteins in rats with streptozotosin-induced diabetes. J. Clin. Invest. **57** : 714-721.
3. **Brunzell, J. D. et al.** 1975. Evidence for diabetes mellitus and genetic forms of triglyceridemia as independent entities. Metabolism **24** : 1115-1121.
4. **Chance, G. W., Albutt, E. C. et al.** 1969. Serum lipid and lipoproteins in untreated diabetic children. Lancet **I** : 1126-1128.
5. **Duncombe, W. G.** 1964. The colorimetric microdetermination of nonesterified fatty acids in plasma. Clin. Chim. Acta **9** : 122-125.
6. **Gonen, B. el al.** 1981. Nonenzymatic glycosylation of low density lipoproteins in vitro. Effects on cell-interactive properties. Diabetes **30** : 875-878.
7. **Greenfield, M. et al.** 1980. Mechanism of hypertriglyceridemia in diabetic patients with fasting hyperglycemia. Diabetologia **18** : 441-446.
8. **Grundy, S. M.** 1979. Transport of very low density lipoprotein triglycerides in varying degrees obesity and hypertriglyceridemia. J. Clin. Invest. **63** : 1274-1283.
9. **Hayes, T. M.** 1972. Plasma lipoproteins in adult diabetes. Clin. Endocrinol. **1** : 247-251.
10. **Murase, T. et al.** 1981. Reciprocal changes, caused by insulin and glucagon, of adipose tissue lipoprotein lipase in rats in vitro. Horm. Metab. Res. **13** : 212-213.
11. **Nikkila, E. A. and Kekki, M.** 1973. Plasma triglyceride transport kinetics in diabetes mellitus. Metabolism **22** : 1-22.
12. **Nikkila, E. A. et al.** 1977. Postheparin plasma lipoprotein lipase and hepatic lipase in diabetes mellitus. Diabetes **26** : 11-21.
13. **Pykalisto, O. J. et al.** 1975. Determinants of human adipose tissue lipoprotein lipase. Effect of diabetes and obesity on basal and diet-induced activity. J. Clin. Invest. **56** : 1108-1117.
14. **Stout, R. W.** 1979. Diabetes and atherosclerosis—The role of insulin. Diabetologia **16** : 141-150.
15. **Taskinen, M. R. and Nikkila, E. A.** 1979. Lipoprotein lipase activity of adipose tissue and skeletal muscle in insulin-deficient human diabetes. Relation to high-density and very-low-density lipoproteins and responce to treatment. Diabetologia **17** : 351-356.
16. **Witzum, J. L. et al.** 1982. Non enzymatic glucosylation of low-density lipoprotein alters its biologic activity. Diabetes **31** : 283-291.